

WHAT IS CLAIMED IS:

1 1. A nucleic acid which comprises a polynucleotide that encodes a fusion  
 2 polypeptide, wherein the fusion polypeptide comprises:  
 3 a) a catalytic domain of a glycosyltransferase; and  
 4 b) a catalytic domain of an accessory enzyme which catalyzes a step in  
 5 the formation of a nucleotide sugar which is a saccharide donor for the glycosyltransferase.

1 2. The nucleic acid of claim 1, wherein the glycosyltransferase is a  
 2 eukaryotic glycosyltransferase.

1 3. The nucleic acid of claim 1, wherein the accessory enzyme is a  
 2 eukaryotic accessory enzyme.

1 4. The method of claim 2, wherein the catalytic domain of the  
 2 glycosyltransferase substantially lacks one or more of a cytoplasmic domain, a signal-anchor  
 3 domain, and a stem region of the glycosyltransferase.

1 5. The nucleic acid of claim 1, wherein the glycosyltransferase is a  
 2 prokaryotic glycosyltransferase.

1 6. The nucleic acid of claim 1, wherein the accessory enzyme is a  
 2 prokaryotic accessory enzyme.

1 7. The nucleic acid of claim 1, wherein the fusion polypeptide further  
 2 comprises a catalytic domain of a second accessory enzyme.

1 8. The nucleic acid of claim 1, wherein the glycosyltransferase is selected  
 2 from the group consisting of sialyltransferases, *N*-acetylglucosaminyltransferases, *N*-  
 3 acetylgalactosaminyltransferases, fucosyltransferases, galactosyltransferases,  
 4 glucosyltransferases, glucuronosyltransferases, xylosyltransferases, and  
 5 mannosyltransferases.

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1                     9. The nucleic acid of claim 1, wherein the accessory enzyme is selected  
2 from the group consisting of:

3                     a GDP-mannose dehydratase, a GDP-mannose 3,5-epimerase, and a  
4 GDP-mannose 4-reductase;

5                     a UDP-glucose 4' epimerase;

6                     a UDP-GalNAc 4' epimerase;

7                     a CMP-sialic acid synthetase;

8                     a neuraminic acid aldolase;

9                     an *N*-acetylglucosamine 2' epimerase;

10                    a phosphate kinase selected from the group consisting of a pyruvate  
11 kinase, a myokinase, a creatine phosphate kinase, an acetyl phosphate kinase, and a  
12 polyphosphate kinase; and

13                    a pyrophosphorylase selected from the group consisting of a UDP-Glc  
14 pyrophosphorylase, a UDP-Gal pyrophosphorylase, a UDP-GalNAc pyrophosphorylase, a  
15 GDP-mannose pyrophosphorylase, a GDP-fucose pyrophosphorylase, and a UDP-GlcNAc  
16 pyrophosphorylase.

1                     10. The nucleic acid of claim 1, wherein the nucleotide sugar is selected  
2 from the group consisting of GDP-Man, UDP-Glc, UDP-Gal, UDP-GlcNAc, UDP-GalNAc,  
3 CMP-sialic acid, GDP-Fuc, and UDP-xylose.

1                     11. The nucleic acid of claim 1, wherein the glycosyltransferase is a  
2 sialyltransferase and the nucleotide sugar is CMP-sialic acid.

1                     12. The nucleic acid of claim 11, wherein the accessory enzyme is a CMP-  
2 sialic acid synthetase.

1                     13. The nucleic acid of claim 11, wherein the accessory enzyme is a  
2 neuraminic acid aldolase or an *N*-acetylglucosamine 2' epimerase.

1                    14. The nucleic acid of claim 1, wherein the glycosyltransferase is a  
2 galactosyltransferase and the nucleotide sugar is UDP-galactose.

1                    15. The nucleic acid of claim 14, wherein the accessory enzyme is a UDP-  
2 glucose 4' epimerase.

1                    16. The nucleic acid of claim 1, wherein the glycosyltransferase is a  
2 fucosyltransferase and the nucleotide sugar is GDP-fucose.

1                    17. The nucleic acid of claim 16, wherein the accessory enzyme is selected  
2 from the group consisting of a GDP-mannose dehydratase, a GDP-mannose 3,5-epimerase, a  
3 GDP-fucose pyrophosphorylase, and a GDP-mannose 4-reductase.

1                    18. The nucleic acid of claim 1, wherein the glycosyltransferase is an *N*-  
2 acetylgalactosaminyltransferase and the nucleotide sugar is UDP-GalNAc.

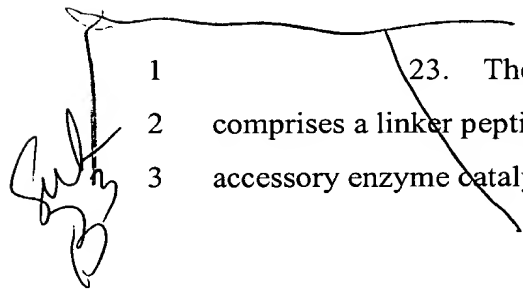
1                    19. The nucleic acid of claim 18, wherein the accessory enzyme is a UDP-  
2 GalNAc 4' epimerase.

1                    20. The nucleic acid of claim 1, wherein the glycosyltransferase is an *N*-  
2 acetylglucosaminyltransferase and the nucleotide sugar is UDP-GlcNAc.

1                    21. The nucleic acid of claim 20, wherein the accessory enzyme is a UDP-  
2 GalNAc 4' epimerase.

1                    22. The nucleic acid of claim 1, wherein the glycosyltransferase is a  
2 mannosyltransferase and the nucleotide sugar is GDP-Man.

1                    23. The nucleic acid of claim 1, wherein the fusion polypeptide further  
2 comprises a linker peptide between the glycosyltransferase catalytic domain and the  
3 accessory enzyme catalytic domain.



1                    24. The nucleic acid of claim 1, wherein the nucleic acid further comprises  
2 a polynucleotide that encodes a signal sequence which is linked to the fusion polypeptide.

1                    25. The nucleic acid of claim 1, wherein the nucleic acid further comprises  
2 a polynucleotide that encodes a molecular tag which is linked to the fusion polypeptide.

1 *Sub B4* 26. An expression vector which comprises a nucleic acid of claim 1.

1 27. A host cell which comprises a nucleic acid of claim 1.

1 28. A fusion polypeptide encoded by a nucleic acid of claim 1.

1 29. A fusion polypeptide that comprises:

2 a) a catalytic domain of a glycosyltransferase; and

3 b) a catalytic domain of an accessory enzyme which catalyzes a step in  
4 the formation of a nucleotide sugar which is a donor for the glycosyltransferase.

1 30. The fusion polypeptide of claim 29, wherein the catalytic domain of the  
2 glycosyltransferase is joined to the carboxy terminus of the accessory enzyme catalytic  
3 domain.

1 31. The fusion polypeptide of claim 29, wherein the glycosyltransferase is a  
2 galactosyltransferase and the accessory enzyme is a UDP-glucose 4' epimerase.

1 32. The fusion polypeptide of claim 29, wherein the glycosyltransferase is a  
2 sialyltransferase and the accessory enzyme is a CMP-sialic acid synthetase.

1 *Sub B5* 33. A method of producing a fusion polypeptide that comprises:

2 a) a catalytic domain of a glycosyltransferase; and

3 b) a catalytic domain of an accessory enzyme which catalyzes a step in  
4 the formation of a nucleotide sugar which is a donor for the glycosyltransferase;

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5 wherein the method comprises introducing a nucleic acid that encodes  
6 the fusion polypeptide into a host cell to produce a transformed host cell; and culturing the  
7 transformed host cell under conditions appropriate for expressing the fusion polypeptide.

1 34. The method of claim 33, wherein the fusion polypeptide is purified  
2 following its expression.

1 35. The method of claim 33, wherein the host cell is permeabilized  
2 following expression of the fusion polypeptide.

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